Italian Botanist 18: 193–214 (2024)
doi: 10.3897/italianbotanist.18.143333
https://italianbotanist.pensoft.net

RESEARCH ARTICLE

Italian Botanist
Published by Società Botanica Italiana

Good practices for the industrial cultivation of the cyanobacterium *Arthrospira platensis* under a greenhouse in a temperate zone (northern Italy)

Rossella Pistocchi[1,2], Franca Guerrini[1], Silvana Vanucci[3], Lorenzo Montalbetti[1], Mara Simonazzi[1], Matilde Mazzotti[4], Federica Abbondanzi[4], Guido Emiliani[4], Laura Pezzolesi[1,2]

1 *Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Via Sant'Alberto 163, 48123 Ravenna, Italy* **2** *Interdepartmental Center for Industrial Research - Renewable Resources, Environment, Sea and Energy (CIRI FRAME), University of Bologna, Via Sant'Alberto 163, 48123 Ravenna, Italy* **3** *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (ChiBioFarAm), University of Messina, Viale Ferdinando d'Alcontres 31, 98166 Messina, Italy* **4** *Micoperi Blue Growth, via Trieste 279, 48122 Ravenna, Italy*

Corresponding author: Rossella Pistocchi (rossella.pistocchi@unibo.it)

Academic editor: Lorenzo Peruzzi | Received 2 December 2024 | Accepted 10 December 2024 | Published 18 December 2024

Citation: Pistocchi R, Guerrini F, Vanucci S, Montalbetti L, Simonazzi M, Mazzotti M, Abbondanzi F, Emiliani G, Pezzolesi L (2024) Good practices for the industrial cultivation of the cyanobacterium *Arthrospira platensis* under a greenhouse in a temperate zone (northern Italy). Italian Botanist 18: 193–214. https://doi.org/10.3897/italianbotanist.18.143333

Abstract

Arthrospira, *Spirulina*, and *Limnospira* are cyanobacteria widely known as food supplements or additives and cultivated worldwide under the commercial name of spirulina. Many studies have been focused on the improvement of operational conditions for optimizing cell growth and harvesting. At present, greater attention is paid to obtaining a good-quality, possibly food-grade, product that can be added to different food formulations and to reducing the environmental impact by saving water and avoiding or minimizing the release of mineral salts in the environment. A few studies have addressed these aspects although, in most cases, through laboratory experiments or pilot plants. This study focused on the effects of medium recycling, monitored at each harvesting step, and nutrient replenishment on *Arthrospira platensis* growth, biomass, biochemical composition, and extracellular polysaccharide (EPS) production, in a production plant located in a temperate zone (northeastern Italy). Four out of the seven largest ponds (11,000 L each) of the plant were followed for a three-month semi-continuous cultivation, which included the period with the highest biomass productivity. Recycling the culture medium after biomass harvest effectively allowed the best nutritional status of the cells. Biomass productivity increased from June to August 2023

Copyright *Rossella Pistocchi et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(mean values: 0.029 and 0.038 g L^{-1} d^{-1} at 25–26 °C and 27–29 °C, respectively). Protein, polysaccharides, and C-phycocyanin contents were 62, 22, and 12%, respectively, while EPS was < 7 mg L^{-1}. The biochemical composition did not vary during the cultivation period, differently from previous studies performed with small culture volumes and for a short time.

Keywords
Arthrospira platensis, nutrient recycling, biochemical composition, extracellular polysaccharides, biomass quality, waste prevention

Introduction

Among the cyanobacteria, some genera (e.g., *Arthrospira*, *Spirulina*, and *Limnospira*) possess helically-coiled trichomes as a prominent diacritical feature. They are widely used as food supplements or food additives under the commercial name of spirulina, which is one of the most widely consumed microalga in large-scale production since the 1970s. Spirulina biomass contains a high quantity of proteins (60–70% on a dry weight basis) with all essential amino acids and is rich in vitamins, dietary minerals, and many bioactive molecules including, among others, essential fatty acids, and pigments (e.g., C-phycocyanin) (Soni et al. 2017).

Spirulina thrives naturally in alkaline waters, rich in minerals, at temperatures ranging from 35 to 40 °C, and thus its cultivation was initially carried out in outdoor open ponds located in tropical geographic areas. The most widespread cultivations concern the species *Arthrospira platensis* Gomont, and the successful commercial cultivation of this cyanobacterium, compared to other microalgae, is ascribable to its extremophilic characteristics allowing fast and strong growth both under high pH values (i.e., pH 9–11), which minimize the risk of biological contamination, for example by protozoa, bacteria, fungi, other microalgae (AlFadhly et al. 2022a; Bumandalai et al. 2024), and under high temperature (optimal cell growth temperature: 35–38 °C; Belay 2013). In the early 1990s, China became the biggest producer of Spirulina biomass, however, concerns arose for the safety of cultures exposed to weather variations and contamination, especially in the absence of appropriate controls and certifications (Belay 2008; Bumandalai et al. 2024), due to dust, microorganisms (microalgae, bacteria, fungi, viruses), protozoa, small animals, and other material (insect fragments, rodent hair, feather fragments).

In the last decade, many studies on spirulina bioactive molecules, secondary metabolites and their recognized therapeutic properties (e.g., antioxidant, anti-inflammatory, immunomodulating, anti-viral, anti-bacterial activities) have renewed interest in its extensive exploitation for application in the nutraceutical, animal feed, cosmetics, and pharmaceutical fields worldwide (AlFadhly et al. 2022b; Dinicolantonio et al. 2020; Ragusa et al. 2021), with an estimated market value of $629.63 million by 2025 (Priyanka et al. 2023). Concurrently, the European Commission's strategic

policies have focused on the key role of algal biomass production for producing high-quality food-grade proteins with a low carbon footprint, according to the approach of ecological sustainability concerning business development. This renewed interest has extended spirulina cultivation to wider geographical areas, including subtropical and temperate ones.

The most common systems used for *A. platensis* culturing are raceway ponds, and the pilot plants set up for production in subtropical and temperate zones are mainly open ponds placed inside greenhouses, which prevent external contamination, rain intrusion, and mitigate temperature excursion allowing cell growth all year round. Several studies have already reported the optimization of spirulina cultivation in subtropical and temperate areas such as Spain (Jimenez et al. 2003a, b; Guidi et al. 2021), Mongolia (Lu et al. 2011), South Africa (Grobbelaar 2009), South Korea (Kim et al. 2018) and northern Italy (Kurpan et al. 2024). These studies were performed in a real environment, i.e., in open ponds (southern Spain and South Africa) or ponds under a greenhouse (in the Canary Islands and in temperate areas with low winter temperatures, such as Mongolia and northern Italy) having volumes from 2,000 to 125,000 L. The reported trials were mainly aimed at testing the feasibility of the cultivation, by evaluating the responses of the cultures in terms of growth to light and temperature conditions and by verifying biomass productivity and, in some cases, the seasonality. Generally, the results obtained indicate that the cultivation of spirulina in temperate areas is possible, despite the interruption of plant operativity in winter months (Lu et al. 2011) or the addition of a heating system to the greenhouse (Kurpan et al. 2024). Data on real operative plants is scarce and, as indicated by Kurpan et al. (2024), "more pilot- and commercial-scale studies are needed to stimulate the production of spirulina and other microalgae in Europe" and other temperate areas.

In recent years, most studies on spirulina cultivation have focused on: i) obtaining a high-quality food-grade product, suitable for increasing the nutritional value of food formulations while maintaining the economic sustainability of the production process; ii) reducing the impact on the environment due to intensive cultivation, by saving water and avoiding or minimizing the release of mineral salts in the environment (Salehipour-Bavarsad et al. 2024).

Furthermore, reducing the operativity costs is an important issue, especially in geographical areas having suboptimal environmental conditions that do not allow to achieve the maximum productivity. For decreasing operativity costs, one of the most widely applied actions, also in line with the need to decrease the environmental footprint of microalgae cultivation, is the recycling of water and nutrients contained in the medium after removing the cells at each harvesting stage. This is a commonly applied procedure for semi-continuous cultivations ever since the big Earthrise farm was established in California (Belay 2013). In this work, it was reported that this practice can have deleterious effects on the quality of the biomass as the organic matter that remains in the filtered medium may stimulate the growth of opportunistic mixotrophic algae, as well as of bacteria and fungi, and can also have autoinhibitory effects on algal growth (Belay 2013).

Studies addressing the problem of water and nutrient recycling with experiments aimed at evaluating how much this practice may affect biomass quality have been performed mainly with small culture volumes (Depraetere et al. 2015; Li et al. 2022) and in one study also in a large pond (Yuan et al. 2019). The reported results highlighted a consistent reduction in growth rate and protein production, an increase in extracellular polysaccharides (EPS), and culture contamination. However, when growth trials are performed in a real productive environment, the recycling of water and nutrients is strictly necessary. It was calculated that, by re-adding the nitrogen (N) and phosphorus (P) consumed based on their content in the harvested biomass, a 15-fold savings of money compared to the use of fresh medium was obtained (Guidi et al. 2021). In other cases, nutrients were completely re-added (Lu et al. 2011) or either recycled or replaced with fresh medium based on the accumulation of organic matter in the medium (Kurpan et al. 2024).

Suggested solutions, such as subjecting the spent medium to ferric chloride flocculation plus carbon adsorption (Morocho-Jácome et al. 2015) or applying an ultrafiltration step using a 5-μm filter (Han et al. 2025), although promising, were tested only with a 2,000 or 600 mL cultures, respectively and did not appear to be applicable to thousands of liters of medium.

In our opinion, and also as suggested by Kurpan et al. (2024), this subject is not sufficiently covered; in fact, despite the high number of studies performed on spirulina cultivation optimization, there is a lack of information on the temporal dynamics of nutrient consumption in large-scale cultures and, consequently, on the management of their reintroduction when the medium is recycled to prevent a nutrient shortage. This study, therefore, focused on an in-depth monitoring of nutrient (nitrate and phosphate) levels in the medium over three months of semi-continuous cultivation, where medium recycling was performed concurrently with monitoring of cell biomass composition. The cultivation system investigated in the present study has a year-long operativity and is already based on medium recycling, thus allowing to obtain further data for a year-based production. The overall aim of the study was to optimize the nutrient inputs to potentially reduce the use of chemicals and the operativity costs, together with the environmental footprint of the cultivation, without affecting the quality of the biomass.

Methods

Arthrospira platensis strain and culture conditions

The strain used for this study was of Moroccan origin and identified as *Arthrospira platensis* Gomont according to the 16S gene sequencing performed by an external laboratory. For mass cultivation a modified Zarrouk Medium (1966), kept at pH values between 9.7 and 10.3, was used.

Production plant operational conditions

The production plant belongs to the company Micoperi Blue Growth srl (MBG) and is located in Ravenna (NE Italy, 44°42.82'N, 12°23.28'E); it consists of a greenhouse containing 11 raceways, seven of which have a volume of 11,000 L, while four are smaller (2 × 5,000 and 2 × 1,000 L) and utilized to scale up the cultures (total pond area = 458 m^2) (Fig. 1a, b). Twice a year, each pond is filled with medium and inoculated with the *A. platensis* culture growing in one of the backup ponds; the depth of the culture is kept at 150–200 mm depending on the season and mixing is provided by a paddle wheel. The cultures are exposed to natural sunlight; however, the greenhouse has shading systems that are activated, according to the season, when the light intensity, measured on the roof, reaches high values. The only temperature regulation consists of bringing it to lower values in summer through a ventilation opening in the roof.

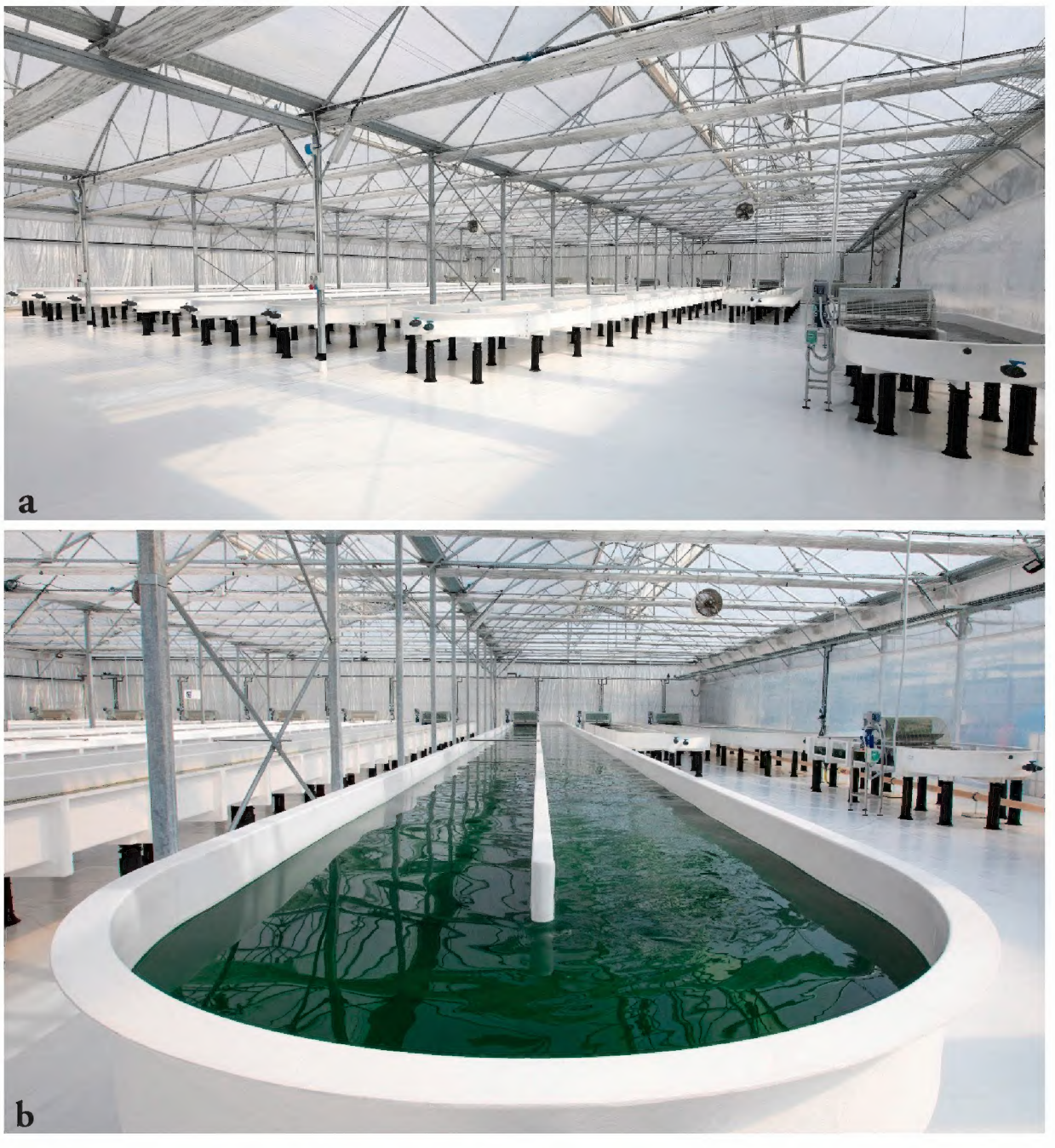

Figure 1. The greenhouse of the Micoperi Blue Growth company where *Arthrospira platensis* is cultivated **a** panoramic view of all the ponds **b** close-up view of *A. platensis* culture.

Annual temperature (min, max, and average) and irradiance values measured inside the greenhouse and on the roof are reported in Figs 2, 3. The ponds operate according to a semi-continuous regime performed by harvesting variable volumes of the culture, based on the biomass concentration measured in each pond. The biomass harvest is performed using a pumping system connected to a vibro-sifter which separates the algal biomass from the liquid medium. The biomass is dehydrated by freeze-drying before its commercialization. Filtered medium is reintroduced into the pond and then reinstated with the main nutrients (N and P) according to the amount of the harvested biomass and to the N and P content of the biomass (around 10 and 0.8%, respectively, as shown below). At the end of the six-month cultivation period, the entire biomass is harvested, followed by the complete elimination of the culture medium. Subsequently, the empty ponds are washed and sanitized and then the production, which is carried out throughout the year, is resumed.

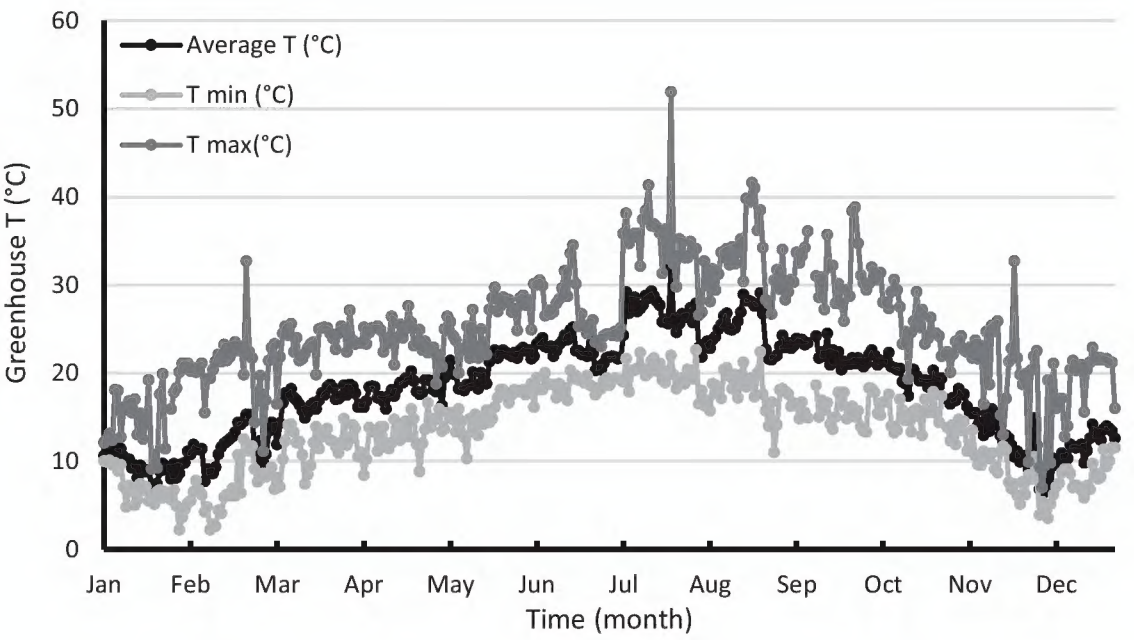

Figure 2. Minimum, maximum, and average temperatures measured inside the greenhouse in the year 2023.

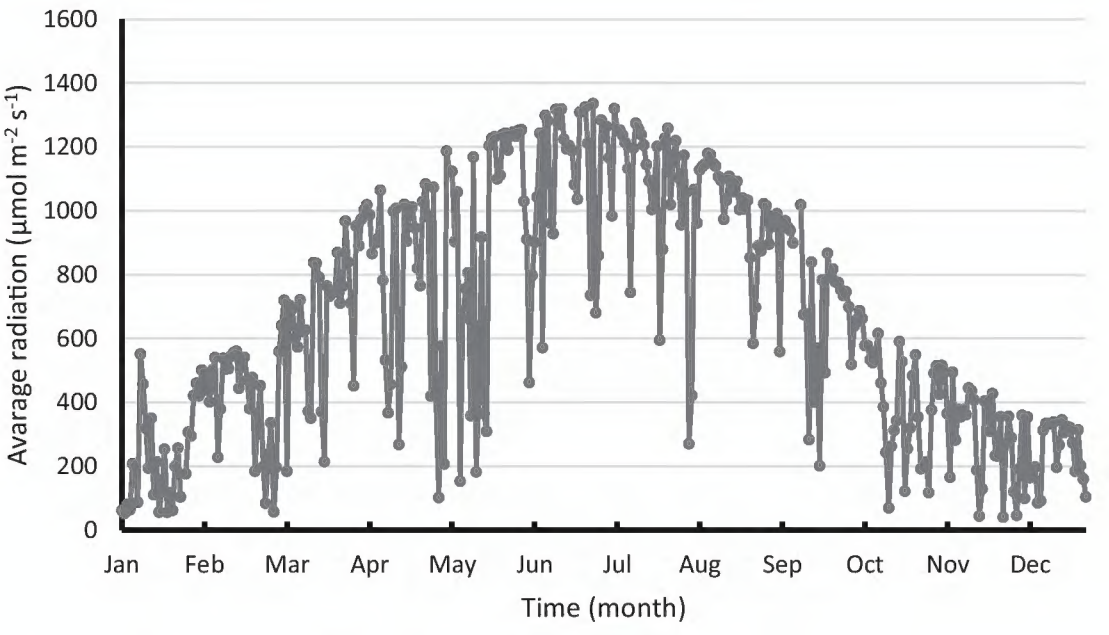

Figure 3. Irradiance measured on the greenhouse roof in the year 2023.

Study procedure

For this study the ponds monitored consisted of four out of the seven larger raceways included in the production plant of MGB. The 4 ponds were monitored between the 10th of May and the 2nd of August 2023, corresponding to the beginning of a new culturing period, and the harvest was performed weekly or bi-weekly at all ponds (Table 1).

Dry weight determination

In each pond, starting from the inoculation day (day 0) the biomass dry weight (DW) was determined every day, in the morning, measuring the absorbance at 750 nm using a spectrophotometer (HACH Lange DR1900) to assess the productivity and the optimal biomass harvesting time. The data were then converted to g DW L^{-1} based on a comparison with DW measurement, which is periodically performed as follows: culture sub-samples (0.02 L) were filtered through pre-weighed glass fiber filters (Whatman GF/C, diameter 47 mm, 1.2-μm pore size); then the filters were washed with a double volume (40 mL) of ammonium formate (0.5 M) according to the Zhu and Lee (1997). Finally, the filters were dried at 105 °C for at least 1 h or until constant weight. Algal biomass dry weight was calculated following the equation:

$$DW(g\,L^{-1}) = \frac{W_t(g) - W_0(g)}{V(L)}$$

where Wt and W_0 are the weight (g) of the filter after drying and before sample filtration, respectively, while V is the volume of filtered culture (L).

Table 1. *Arthrospira platensis* cultivation period followed in the present study. All the days in which the nutrient concentrations were analyzed, as well as harvesting days, are indicated.

Date	Days	Harvesting days
10/05/2023	0	
30/05/2023	20	Yes
01/06/2023	22	
06/06/2023	27	Yes
08/06/2023	29	
13/06/2023	34	Yes
15/06/2023	36	
21/06/2023	42	Yes
23/06/2023	44	
28/06/2023	49	Yes
29/06/2023	50	
05/07/2023	56	Yes
07/07/2023	58	
19/07/2023	70	Yes
21/07/2023	72	
02/08/2023	84	Yes

Algal biomass productivity

Volumetric productivity (VP, g L^{-1} day^{-1}) of algal biomass was determined using the following equation:

$$VP = (DW_2 - DW_1) / (t_2 - t_1),$$

where DW_2 and DW_1 are the values of algal biomass (g L^{-1}), t_2 and t_1 is the corresponding period (day).

Areal productivity was determined according to Kim et al. (2018):

Areal productivity (AP, g m^{-2} d^{-1}) = Volumetric productivity (VP) × (Odepth × 1000),

where Odepth is the pond operating depth.

Nutrient analysis

During the three-month experimentation, inorganic nutrients were measured in the medium of the four ponds on each harvest day, and two days after the nutrient recycling/replenishment step to allow a correct mixing of the medium. For the analyses, subsamples of the culture medium were pre-filtered through Whatman GF/C filters (1.2-µm pore size); aliquots of 1 mL were then filtered through a 0.22-µm syringe filter and injected into an ion chromatograph (883 Basic IC plus, Metrohm) equipped with an anionic column (Metrosep A Supp 4—250/4.0) for N and P.

The nutrient uptake rates (U) were calculated according to Lim et al. (2006). Nitrate and phosphate uptake rates (mmol g biomass^{-1} day^{-1}) were calculated from the nutrient concentration in the medium (C) and the change in algal biomass (γ) over a period (Δt), when the depletion of nutrients was linear, and no replenishment was performed. Δt, therefore, differs depending on nutrient sources and algal conditions. The following equations were used:

$$U = -\frac{C_t - C_o}{\gamma \ \Delta\tau}$$

$$\gamma = \frac{B_t - B_0}{\ln B_t - \ln B_0},$$

where B_t and B_0 are *A. platensis* dry weight biomasses (g L^{-1}) at time t and 0, respectively.

Protein extraction and analysis

For this and successive intracellular analyses, *A. platensis* biomass was harvested by filtration (11-µm pore size). Protein extraction was performed on freeze-dried algal biomass (about 10 mg). The samples were extracted with 3.0 mL of NaOH (0.5 M)

and incubated at 90 °C for 15 min, then transferred on ice for 2 min and subsequently centrifuged (2,250 × g, 10 min). The resulting supernatant was collected, and the extraction process was repeated three times. Protein content was determined on the collected supernatant with the Folin phenol reagent according to Lowry's method (1951).

Intracellular polysaccharide extraction and analysis

Intracellular polysaccharides were extracted from freeze-dried samples (5–10 mg), digestion was carried out using sulfuric acid 80% at 37 °C for 60 min, then they were quantified spectrophotometrically (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) through the Phenol Sulfuric Method (DuBois et al. 1956; Kochert 1973) using glucose as standard.

C-phycocyanin analysis

To extract C-phycocyanin (C-PC), freeze-dried aliquots of *A. platensis* biomass were weighed (30 mg), then 2 mL of 0.01 M sodium phosphate buffer (pH 7.4) was added. The samples were incubated at 25 °C for 24 hours with agitation. At the end of the incubation period, tubes were centrifuged for 20 min at 2,500 × g. Subsequently, the supernatant was collected and diluted 1:20 with the previously used extraction buffer and centrifuged. The absorbance of the supernatant was measured with a spectrophotometer (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) at 615 nm, and 652 nm. The concentration of C-phycocyanin was calculated according to the equation of Bennett and Bogorad (1973) reported in Pan-uai et al. (2018).

Cell elemental composition

Carbon (C) and N cell contents were determined using 2–4 mg of freeze-dried algal biomass. The analysis was conducted using a ThermoFisher organic elemental analyzer (Flash 2000) set for CHNS–O determination with a copper/copper oxide column. The standard 2,5-bis-(5-tert-bu-tyl-2-benzooxazol-2-yl) thiophene (BBOT) was used for calibration.

(P cell content was determined following Menzel and Corwin (1965): 10 mg of freeze-dried algal biomass was digested with a solution of 5% potassium persulfate in an autoclave at 121 °C for 30 min. Total P was then determined spectrophotometrically (UV/VIS/NIR, JASCO V-650, Tokyo, Japan) by using the ascorbic acid method at 885 nm.

Contaminants control

The amounts of bacteria, molds, and yeasts were evaluated by an external laboratory through colony-forming unit (CFU) counts, as part of the quality control of the company which is performed on each fresh batch of harvested biomass. The presence of other microorganisms (cyanobacteria, microalgae, zooplankton) was checked weekly by observing culture aliquots under an optical inverted microscope (Zeiss Axiovert 100).

Extracellular polysaccharides analysis

Extracellular polysaccharides (EPS) were extracted from culture medium filtered through Whatman GF/C filters, following the Myklestad and Haug protocol (1972). Two volumes of absolute ethanol were added to one volume of culture and stored at -20 °C for 24 h. The solution was centrifuged for 25 min at 14,700 × g at 4 °C and the pellet was used to measure polysaccharide content. Carbohydrate digestion was carried out using 80% sulfuric acid at room temperature for 20 h; total carbohydrate content of the medium was determined by the phenol sulfuric method (DuBois et al. 1956; Hellebust and Craigie 1973) using glucose as standard.

Statistical analysis

Differences among samples in nutrient (N and P) concentrations (and cellular composition over time were tested by multivariate analysis of variance (ANOVA) using PAST 4.11 software (Hammer and Harper 2001). Additionally, Pearson's correlation coefficient between productivity and temperature was calculated. A significance threshold of $P < 0.05$ was applied to all the analyses.

Results

Arthrospira platensis growth and productivity

Growth patterns of *A. platensis* biomass and of the temperature average measured in the pond medium for the entire period considered (in total 84 days) are reported in Fig. 4 which highlights that during the study period, three sub-periods (i.e., temporal phases) were observed in terms of temperature variation and its increasing values. The first sub-period, from day 0 to the first biomass harvest performed on day 20, was characterized by the lowest recorded temperature values (i.e., 23–24.5 °C) and by a steady growth of *A. platensis* cultures with a biomass concentration increasing from 0.190 ± 0.004 g L^{-1} to 0.400 ± 0.036 g L^{-1}. In the second period, from day 20 to day 40, temperature slightly increased with average values never exceeding 26 °C, still resulting in a slow growth of the cultures so that harvesting was performed weekly and it consisted of the collection of culture volumes equal to 1/6 of the total pond volumes, after which constant biomass values of 0.395 ± 0.011 g L^{-1} were reached. In the third period (from day 42 to day 84), concomitant to the temperature increase (between 27 and 29 °C), a further increase in biomass concentration was observed (0.461 ± 0.003 g L^{-1}) allowing the harvest of higher culture volumes (1/4 of the total pond volume) either weekly (on days 44 and 56) or biweekly (on days 70 and 84). Similarly to the second period, after each harvest *A. platensis* cultures resumed biomass values comparable to the preceding ones (0.457 ± 0.021 g L^{-1}).

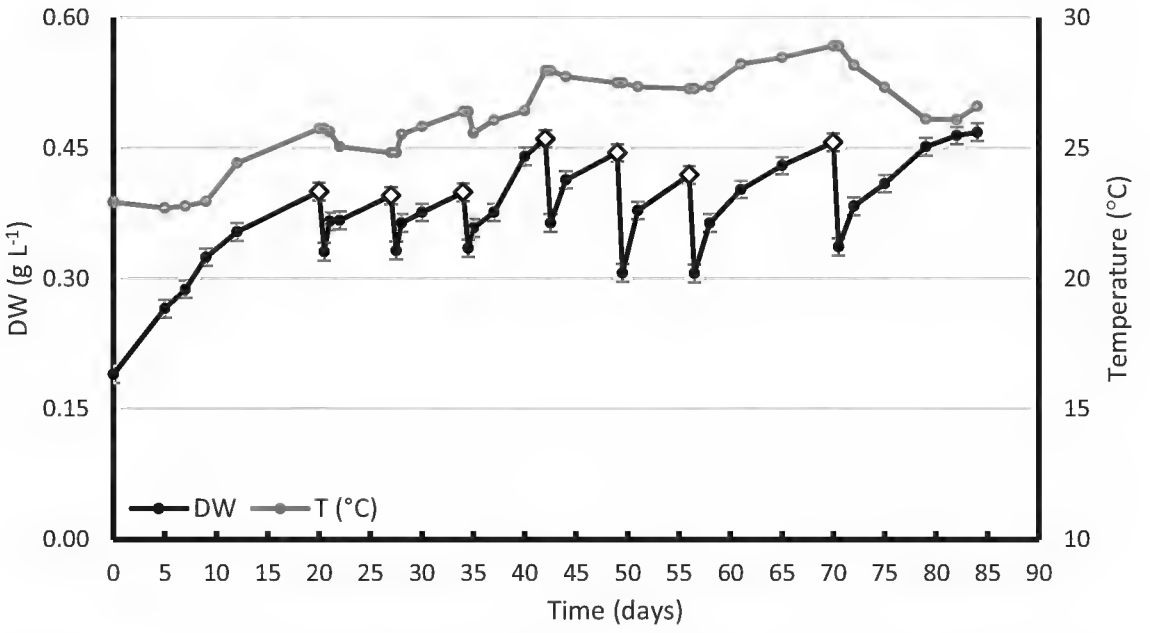

Figure 4. Growth pattern of *Arthrospira platensis* cultivation from day 0 to day 84. Biomass is expressed in terms of mean dry weight (DW, g L^{-1}) for the four ponds. Temperatures represent the average values measured in the culture medium (°C). Harvesting days are indicated with white symbols.

During the first 20 days, biomass productivity was 0.011 g L^{-1} d^{-1}; in the second and third temporal phases, it increased by 2–3-fold (0.029 ± 0.005 and 0.038 ± 0.008 g L^{-1} d^{-1}, respectively), showing a significant positive correlation with temperature ($P < 0.05$). The maximum volumetric productivity value of 0.038 g $L^{-1}d^{-1}$, measured during the study period, corresponded to an areal productivity of 7.6 g m^{-2} d^{-1}.

Nutrient concentrations in the culture medium

Nitrate and phosphate concentrations in the culture medium measured during the cultivation time are shown in Fig. 5, where values registered immediately after the harvesting day indicate the nutrient levels following the medium recycling/replenishment process. Nitrate concentrations showed a decrease during the initial period of biomass growth (uptake rate: 0.219 mmol g^{-1} day^{-1}). Thereafter, for four weeks, during the semi-continuous operativity of the ponds, concentrations remained at values very similar to those initially added; subsequently, a small, but not statistically significant (ANOVA, $P > 0.05$), increase was observed from the initial value of 860.51 to the final one of 937.74 mg L^{-1}. Phosphate concentrations showed a decreasing trend until day 20 (uptake rate: 0.010 mmol g^{-1} day^{-1}); starting from day 44, they displayed an opposite trend compared to nitrates with a significant decrease from the initial value of 79.21 to the final one of 55.90 mg L^{-1} (ANOVA, $P < 0.05$). As a consequence, the N/P molar ratio in the medium remained at a constant value near the Redfield value of 16 until the end of June (day 49); subsequently, it increased up to a maximum of 25.8 at the end of the study period (Fig. 6).

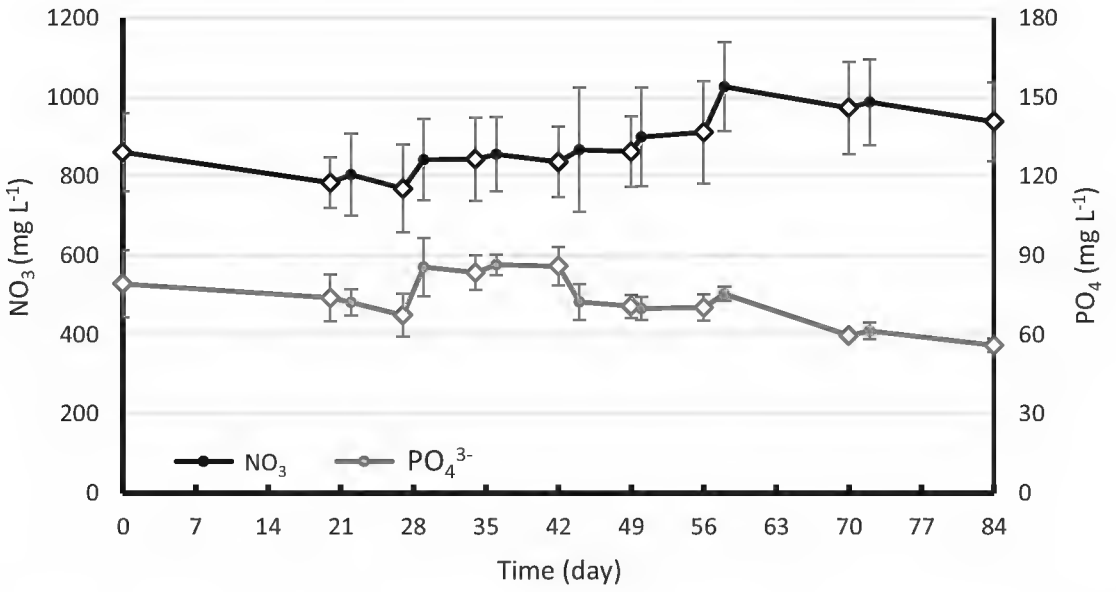

Figure 5. Nitrate and phosphate concentrations (mg L^{-1}) measured in the culture medium during the growth of *Arthrospira platensis*. White lozenge symbols indicate the nutrients measured before the harvesting, the round symbols (black for nitrate, grey for phosphate) represent the nutrients measured two days after the reintegration procedure.

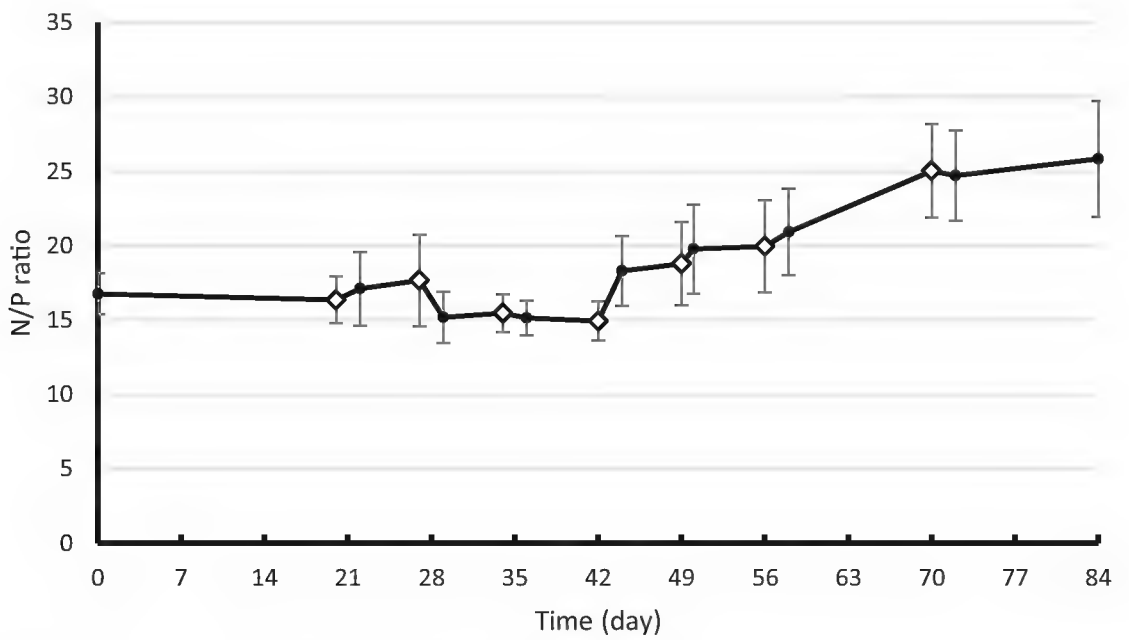

Figure 6. Molar N/P ratio in *Arthrospira platensis* culture medium.

Cellular carbon, nitrogen, and phosphorus

Carbon, nitrogen, and phosphate contents in *A. platensis* cells were assessed in all harvested biomass lots. Like for proteins, polysaccharides, and C-phycocyanin (see below), values at day 0 were not recorded as the biomass analysed on that day was derived from ponds with different characteristics. Fig. 7 shows the constancy of the three elements throughout the entire study period, with no significant differences among ponds and harvesting days (ANOVA, $P > 0.05$). The average cell contents were 46.10 ± 1.92, 10.30 ± 0.64, and 0.85 ± 0.05% for C, N, and P, respectively. The constant cellular C and N contribution led to a stable trend over time of both C/N and N/P molar ratios (average values: 5.2 ± 0.2 and 27.7 ± 1.2, respectively).

Biochemical composition of the biomass

Fig. 8 shows the average percentage of proteins and polysaccharides in lyophilized spirulina biomass. Total proteins displayed no significant differences among ponds and harvesting days (ANOVA, $P > 0.05$), remaining constant over time (average value: 61.6 ± 0.4%). Differently, the contribution of polysaccharides decreased significantly (ANOVA, $P < 0.05$) over time, ranging from a maximum of 26.44 ± 3.67% to a minimum of 18.25 ± 1.16%. C-phycocyanin (C-PC) content in *A. platensis* biomass was also monitored (Fig. 9) due to its important commercial value. Like proteins, no significant changes were found during the study period (ANOVA, $P > 0.05$), with an average contribution of 12.24 ± 0.17%.

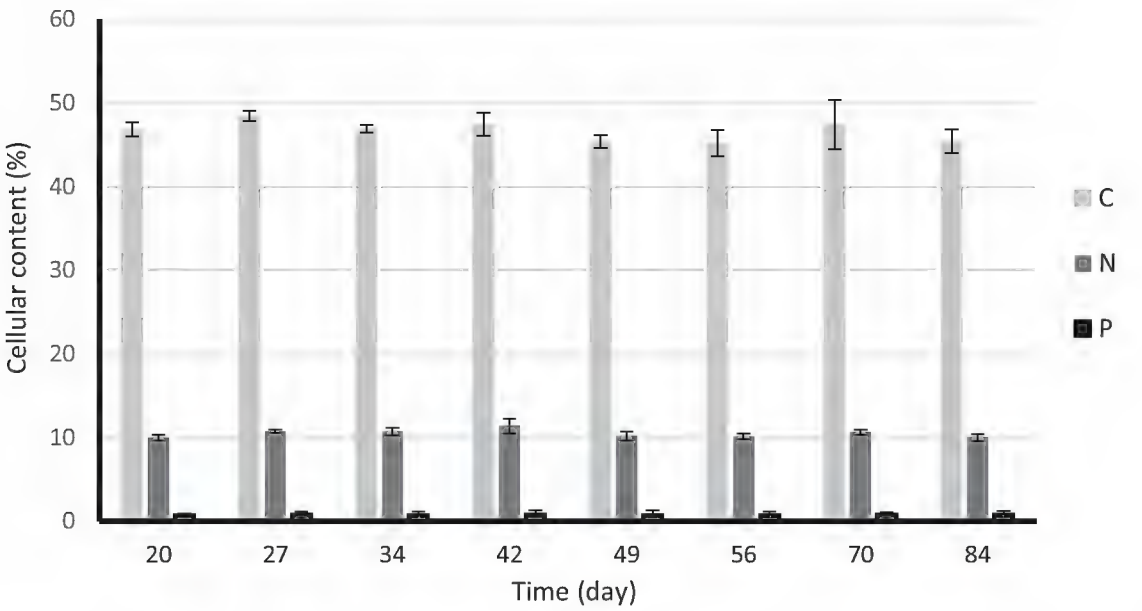

Figure 7. Cellular carbon, nitrogen and phosphorous content in the harvested *Arthrospira platensis* biomass.

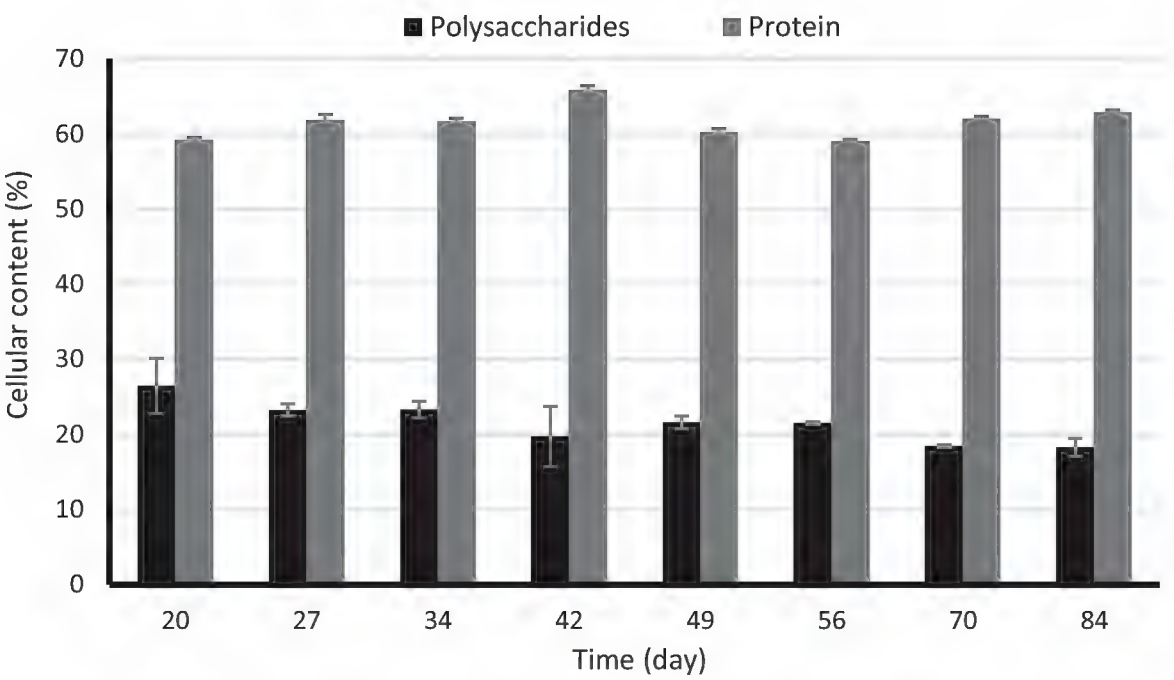

Figure 8. Cellular content (%, on dry weight) of polysaccharides and proteins in the harvested *Arthrospira platensis* biomass.

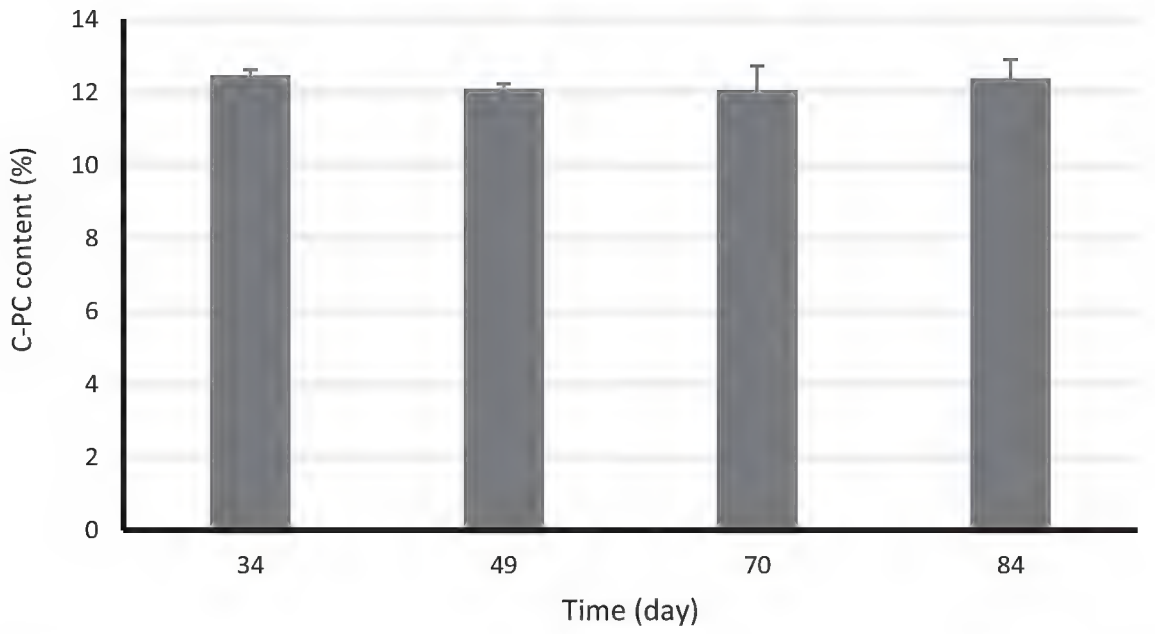

Figure 9. Cellular content (%, on dry weight) of C-phycocyanin in the harvested *Arthrospira platensis* biomass.

Extracellular polysaccharides and contaminants

As shown in Fig. 10, EPS concentration in the ponds at the first harvest was low (ca. 2 mg L^{-1}); subsequently, a slight increase was observed, after which EPS levels remained within a constant range of 5–7 mg L^{-1} for the entire period.

In order to assess food-grade quality of the cultivated *A. platensis*, each fresh biomass batch harvested was analysed for the presence of bacteria, molds, and yeasts, and their abundances were very low (Table 2). Moreover, opportunistic microalgae or zooplankton were rarely found in the biomass during this study (Fig. 11).

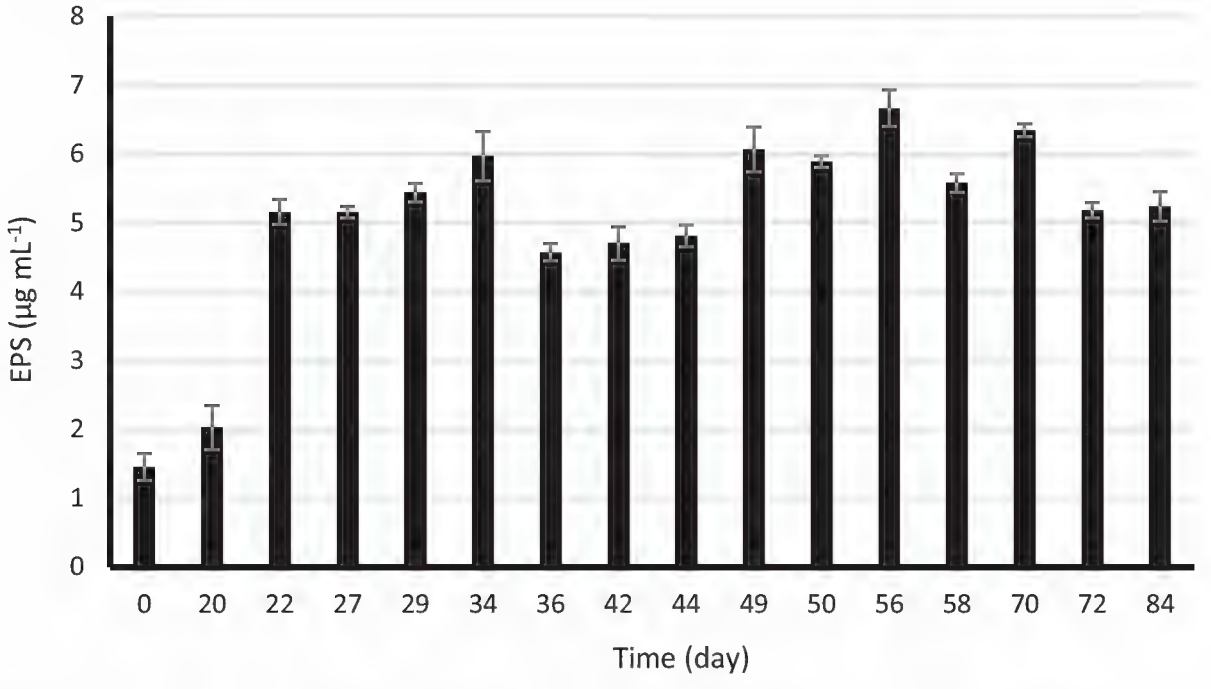

Figure 10. Concentration of extracellular polysaccharides (EPS, mg L^{-1}) in the culture medium of *Arthrospira platensis* for all the growth period considered.

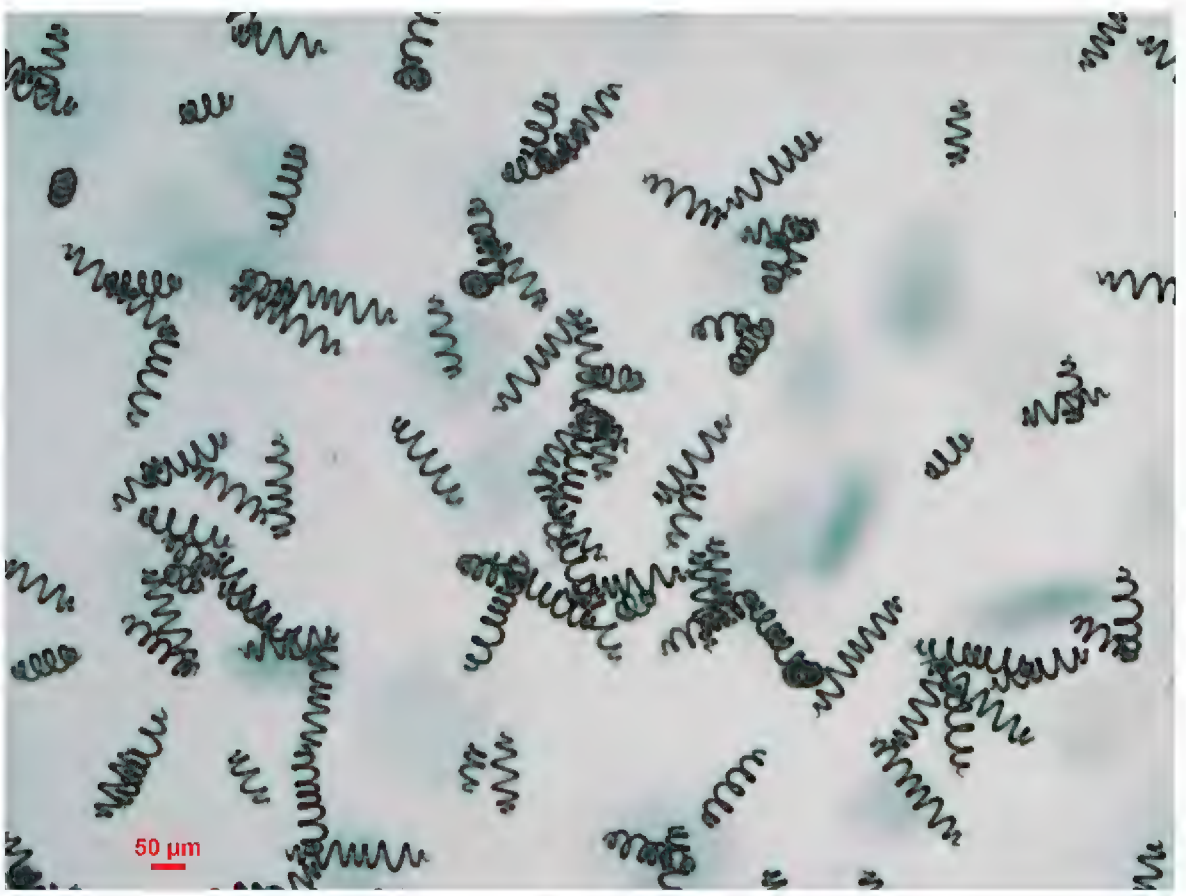

Figure 11. *Arthrospira platensis* culture under the microscope (320×).

Table 2. Contaminants in the fresh biomass batches harvested and analysed in the present study. The biomass from the different ponds was combined in a single batch and analysed.

DAY	BACTERIA	MOLDS	YEASTS
		(CFU g FW biomass^{-1})	
20	$7.8*10^3$	<40	<10
27	$4.8*10^3$	<40	<10
34	$2.5*10^3$	<150	<10
42	$8.0*10^3$	<40	<10
49	$1.7*10^3$	<10	<10
56	$1.2*10^3$	<10	<10
70	$2.7*10^3$	<10	25

Discussion

Over the study period, the combination of high irradiance and temperature values of 27–29 °C led to maximum productivity from mid-June till the beginning of August. Although *Arthrospira platensis* has an optimal temperature growth range of 35–38 °C, with variability among strains (Belay 2013; AlFahdly et al. 2022a), it can grow under a wider temperature range (20–37 °C, Soni et al. 2017) and values around 30 °C or lower have been reported to promote spirulina growth (e.g., Trabelsi et al. 2009; AlFadhly et al. 2022a).

The maximum volumetric productivity reached a value of 0.038 g $L^{-1}d^{-1}$. It corresponded to an areal productivity of 7.6 g m^{-2} d^{-1}, in line with results reported for various productive plants located in Mongolia (range 5.29–9.09 g m^{-2} d^{-1}; Lu et al. 2011), while it was higher than the one observed in the Canary Islands in ponds of 8000 L

(6 g m^{-2} day^{-1}, Guidi et al. 2021) and lower than those obtained in southern Spain and Italy in very large ponds during summer (450 m^2: 14 g m^{-2} d^{-1}, Jimenez et al. 2003a; 300 m^2: 16 g m^{-2} d^{-1}, Kurpan et al. 2024). This production variability indicates that, besides the geographical location, the size of the culture system and/or its operativity highly influence the performance of the plant, as previously reported by Guieysse and Plouviez (2024), and that the industrial production in temperate zones is feasible with a year-long operativity, even without a heating system, as in northern Italy (Kurpan et al. 2024). The cultivation system of this study, in fact, has a year-long operativity, and the company's historical data attest that the highest productivity values span over a wider period (i.e., from June to October), while they decrease by 25% from February to May (T range: 16–24 °C), reaching the lowest values in December and January, (T range: 8.7–11.2 °C), which correspond to a 65% decrease (data not shown). At this stage, cells still appear healthy and, as soon as the photoperiod and temperature increase, cell growth resumes shortly. While this implies low productivity for a few months, conversely it avoids downtime due to pond emptying and, presumably, ensuring a well-timed turnover of the cultures.

The harvesting approach applied in this study, consisting of the collection of variable and low culture volumes with respect to the pond volume (from 1/6 to 1/4 of the total volume) and based on the biomass concentration in the ponds, ensured that the biomass in the ponds remained in the range 0.300–0.460 g L^{-1}, preventing its exposition to unsuitable irradiance levels (either too high or low). This is consistent with a previous study on repeated batch cultivation (Randmann et al. 2007) showing an optimal growth rate with a blend biomass concentration of 0.400 g L^{-1}, and also with cultivation strategies in larger ponds (Grobbelaar 2008).

Nitrate and phosphate concentrations were strictly monitored to evaluate the accuracy of the nutrient recycling strategy. Replenishment only of the presumably consumed nutrients after each harvest would be expected to maintain stable nutrient concentrations for the entire duration of the cultivation period. In fact, nitrates did not significantly change, whereas the decrease in phosphate concentration in the culture medium could be due to salt precipitation with the progression of biomass growth, as previously reported (Lodi et al. 2003). In addition, deviations of the C/N and N/P cellular ratios from the Redfield ratio (i.e., 6.6 and 16, respectively) could be ascribed to the high N content commonly reported for this species (Guo et al. 2022). The slow nutrient uptake observed, together with the slow consumption of the added carbonate (data not shown), clearly indicate that the recycling of the residual medium from the harvested biomass is an important issue both from an economic point of view and for avoiding the release of nitrogenous compounds in the environment (Depraetere et al. 2015).

A. platensis has important nutritional properties largely due to its high protein content (e.g., Soni et al. 2017), reported in various studies as being between 55 and 70% DW. Repeated reuse of the culture medium was reported to cause a significant protein content decrease from 35.8 to 26.7% (Depraetere et al. 2015) or from 38.64 to 14.25% (Andrade et al. 2019); in these studies, C-PC, which has important commercial applications (Lafarga et al. 2020) and also plays a secondary role as intracellular N storage compounds, also decreased like other pigments, while cellular sugars increased as a response to nutrient depletion. The percentages of proteins and C-PC

reported for our cultures remained constant over time; proteins showed an average value of 61.6% which is similar to the average value measured for one year by MBG (65,9%, data not shown) and it is consistent with the protein content reported for most of the commercialized spirulina products (Priyanka et al. 2023). C-PC also had an average value (12.24%) consistent with that found by Xie et al. (2015), i.e., 11% of the total biomass, in spirulina grown under fed-batch conditions with a nitrate concentration of 10 mM. Starting values of storage polysaccharides in the studied cultures are in accord with the range of 13–25% DW previously reported (Priyanka et al. 2023), although this amount is variable depending on the culture medium used and the irradiance available for the cells (Habib et al. 2008). The decrease observed in the last period of monitoring could be linked to the presence of nitrates as the only N source for the cultures; in the absence of ammonium, as in this case (data not shown), the highest consumption of reducing equivalents was observed (Flores and Herrero 2005), in which stored polysaccharides might be involved and utilized. This is also the reason why the presence of C in the medium is one of the most important factors for the optimum growth of this organism and its enzyme activities (Chauhan et al. 2012).

Results of this study clearly highlighted that the biochemical composition of the biomass remained constant despite recycling of the medium after each harvesting step. Previous studies reported that the protein content of the biomass was not affected only through the application of an ultrafiltration method to the medium before its reintroduction in the cultivation system (Han et al. 2025). Most of the studies on the effect of medium recycling on spirulina growth and quality, however, were performed using small vessels and running few cultivation cycles; only a few similar studies were performed in industrial systems, where the recycling of the medium was applied for short periods: one month in the Canary Islands (Guidi et al. 2021) or for periods defined by the increase in extracellular organic matter (Kurpan et al. 2024). This study is the first one that applied this operativity for a long time in a commercial plant.

EPS released from *A. platensis* cells can also provide important information on culture health; in addition, they can severely affect harvesting efficiency by reducing medium filterability. Although this parameter is not often monitored in cultivation systems, the values in our cultures were by far lower than those reported for fresh medium (range: 14–40 mg L^{-1}) (Depraetere et al. 2015; Li et al. 2022). Several factors can influence the release of polysaccharides in the culture medium as a means to protect the cyanobacteria; a study by Phélippé et al. (2019) emphasizes the impact of high and low temperatures, nutrient deficiency, high salinity, and particularly high light intensity. Trabelsi et al. (2009) found that the interaction between temperature and light intensity had a strong influence on EPS concentration in spirulina batch cultures, while when growth conditions were favourable EPS production was low. A further stress factor that could influence EPS synthesis is the recycling of the culture medium. To the best of our knowledge, there are no reports on EPS monitoring in large-scale systems. For small volumes, Li et al. (2022) reported 1.75 times increase in EPS concentration from the first to the third recycling, and, as reported above, concentrations were higher (range: 14 to 22.5 mg L^{-1}) than the ones observed in this study. In Depraetere et al. (2015) extracellular sugars were ca. 40 mg L^{-1} when only fresh medium was used and gradually increased to 200 mg L^{-1} during 45 days

of recycled medium addition. In both studies, the recycled medium was applied to a culture volume of about 1 L, indicating that the number of stressors triggering EPS in a small volume is higher than in large ponds where spirulina is in a more favourable environment, similar to that in which spirulina blooms under natural conditions. The low EPS values observed throughout the analyzed period were consistent with the observation that the ponds were regularly free of foam and cells retained their healthy shape (i.e., normal length and spiral form). This, together with the low amounts of contaminants recorded, which were in line with measurements that have been made over the last seven years by the MBG company (data not shown), attest to the good practices used throughout the cultivation process and are in agreement with previously published data (Jimenez et al. 2022a; Guidi et al. 2021). Only on one occasion, following mechanical rupture of the sieving machine, an increase in EPS levels (20–25 mg L^{-1}) and the presence of organic matter flocs in the medium with an enhancement in bacteria numbers (Zerlotin 2021) were observed. During such an event, the presence of opportunistic microalgae or zooplankton consequently increased. Given the lack of a directive for algal biomass, documentary research on similar food products and the analysis of the company's historical data allowed to fine-tune a microbiological values guide to be followed for monitoring the quality of the harvested product. For the administrative region of Emilia-Romagna (where MBG is located), the limit for ready-to-eat vegetables is 1.0×10^6 CFU for total mesophilic bacteria and 1.0×10^2 CFU g^{-1} for molds and yeasts (Regione Emilia-Romagna 2009–2011), which are an order of magnitude higher than those reported here for *A. platensis* biomass.

Conclusions

Weekly monitoring of the medium and *Arthrospira platensis* biomass, collected from four ponds in the productive industrial plant (MBG) located in a temperate zone proved that it is possible to recycle the medium for some months without affecting the quality of the microalga. This was attested by the good biochemical composition of the biomass and the low presence of organic substances and contaminants in the medium. This can be attributed to an appropriate management of the system, considering both operation mode and operational conditions, including a moderate mixing of the ponds, a stringent monitoring of the environment parameters, and a gentle harvesting procedure. These good practices adopted are also testified by the fact that the four ponds showed similar values for each measured parameter, and it is noteworthy that this occurred in a temperate zone with high climate variability. The results of this study allow to conclude that a better operativity in terms of nutrient recycling could be adopted to decrease the level of nutrients in the medium and facilitate its disposal as a waste at the end of the six-month cultivation period. It is thus possible to envisage a solution consisting of gradually decreasing the re-addition of nutrients during the last harvesting steps (e.g., in the last month of the culturing period). Further studies performed at an industrial plant could be aimed at testing if a decrease of nutrient replenishment could be performed without affecting the quality of the biomass leading to economic savings and an improvement in the environmental footprint of the cultivation, thus achieving a more sustainable process.

Author contributions:

Rossella Pistocchi: Conceptualization, Project administration, Resources, Validation, Visualization, Writing – original draft. Franca Guerrini: Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – review and editing. Silvana Vanucci: Methodology, Validation, Visualization, Writing – review and editing. Lorenzo Montalbetti: Data curation, Formal analysis, Visualization, Methodology, Investigation, Writing – review and editing. Mara Simonazzi: Data curation, Formal analysis, Visualization, Methodology, Investigation, Writing – review and editing. Matilde Mazzotti: Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – review and editing. Federica Abbondanzi: Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Writing – review and editing. Guido Emiliani: Data curation, Funding acquisition, Project administration, Writing – review and editing. Laura Pezzolesi: Conceptualization, Resources, Validation, Visualization, Writing – review and editing.

References

AlFadhly NKZ, Alhelfi N, Altemimi AB, Verma DK, Cacciola F (2022a) Tendencies affecting the growth and cultivation of genus *Spirulina*: An investigative review on current trends. Plants 11: 3063. https://doi.org/10.3390/plants11223063

AlFadhly NKZ, Alhelfi N, Altemimi AB, Verma DK, Cacciola F, Narayanankutty A (2022b) Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A review. Molecules 27: 5584. https://doi.org/10.3390/molecules27175584

Andrade BB, Cardoso LG, Assis D de J, Costa JAV, Druzian JI, da Cunha Lima ST (2019) Production and characterization of *Spirulina* sp. LEB 18 cultured in reused Zarrouk's medium in a *raceway*-type bioreactor. Bioresource Technology 284: 340–348. https://doi.org/10.1016/j.biortech.2019.03.144

Belay A (2008) *Spirulina* (*Arthrospira*): Production and quality assurance. In: Gershwin ME, Belay A (Eds) Spirulina in Human Nutrition and Health. CRC Press, 1–25. https://doi.org/10.1201/9781420052572

Belay A (2013) Biology and industrial production of *Arthrospira* (*Spirulina*). In: Richmond A, Hu Q (Eds), Handbook of Microalgal Culture Applied Phycology and Biotechnology. John Wiley & Sons, Ltd publ., New Deli, India, 339–358. https://doi.org/10.1002/9781118567166.ch17

Bennett A, Bogorad L (1973) Complementary chromatic adaptation in a filamentous blue-green alga. The Journal of Cell Biology 58: 419–435. https://doi.org/10.1083/jcb.58.2.419

Bumandalai O, Bayliss KL, Moheimani NR (2024) Innovative processes for combating contaminants in fresh spirulina. Algal Research 78: 103397. https://doi.org/10.1016/j.algal.2024.103397

Chouhan S, Kaithwas V, Kachouli R, Bhargava S (2012) Productivity of the Cyanobacterium *Spirulina platensis* in cultures using high bicarbonate and different nitrogen sources. American Journal of Plant Physiology 8: 17–31. https://doi.org/10.3923/ajpp.2013.17.31

Depraetere O, Pierre G, Noppe W, Vandamme D, Foubert I, Michaud P, Muylaert K (2015) Influence of culture medium recycling on the performance of *Arthrospira platensis* cultures. Algal Research 10: 48–54. https://doi.org/10.1016/j.algal.2015.04.014

Dinicolantonio JJ, Bhat AG, OKeefe J (2020) Effects of spirulina on weight loss and blood lipids: a review. Open Heart 7: e001003. https://doi.org/10.1136/openhrt-2018-001003

DuBois Michel, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28: 350–356. https://doi.org/10.1021/ac60111a017

Emilia Romagna (2009–2011) Fornitura di derrate e prodotti alimentari. Allegato A al Capitolato Tecnico (rif. Tabella I, insalata IV gamma e verdure pronte al consumo per servizi di ristorazione collettiva pubblica).

Flores E, Herrero A (2005) Nitrogen assimilation and nitrogen control in cyanobacteria. Biochemical Society Transactions 33: 164–167. https://doi.org/10.1042/BST0330164

Grobbelaar JU (2009) From laboratory to commercial production: a case study of a *Spirulina* (*Arthrospira*) facility in Musina, South Africa. Journal of Applied Phycology 21: 523–527. https://doi.org/10.1007/s10811-008-9378-5

Guidi F, Gojkovic Z, Venuleo M, Assunçao PACJ, Portillo E (2021) Long-term cultivation of a native *Arthrospira platensis* (Spirulina) strain in Pozo Izquierdo (Gran Canaria, Spain): technical evidence for a viable production of food-grade biomass. Processes 9(8): 1333. https://doi.org/10.3390/pr9081333

Guieysse B, Plouviez M (2024) Microalgae cultivation: closing the yield gap from laboratory to field scale. Frontiers in Bioengineering and Biotechnology 12: 1359755. https://doi.org/10.3389/fbioe.2024.1359755

Guo D, Wang Y, Gao Y b, Lyu Y, Lin Y, Pan Y, Zhu L (2022) Nitrogen migration in products during the microwave-assisted hydrothermal carbonization of *Spirulina platensis*. Bioresource Technology 351: 126968. https://doi.org/10.1016/j.biortech.2022.126968

Habib MAB, Parvin M, Huntington TC, Hasan MR (2008) A review on culture, production and use of spirulina as food for humans and feeds for domestic animals. FAO Fisheries and Aquaculture Circular 1034: 1–33.

Hammer Ø, Harper DA (2001) Past: paleontological statistics software package for education and data analysis. Palaeontologia Ectronica 4(1): 4.

Han Q, Huang Y, Wang S, Zou Q, Yu B, Luo H, Li C (2025) Improved recyclability of Spirulina culture medium through ultrafiltration. Separation and Purification Technology 354: 129168. https://doi.org/10.1016/j.seppur.2024.129168

Hellebust JA, Craigie JS (1973) Carbohydrate determination by the phenol sulfuric acid method. In: Handbook of Phycological Methods: Physiological and Biological Methods. Cambridge University Press, 95–97.

Jiménez C, Cossìo BR, Niell FX (2003a) Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. Aquaculture 221: 331–345. https://doi.org/10.1016/S0044-8486(03)00123-6

Jiménez C, Cossìo BR, Labella D, Niell XF (2003b) The feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain. Aquaculture 217: 179–190. https://doi.org/10.1016/S0044-8486(02)00118-7

Kim T, Choi W-S, Ye B-R, Heo S-J, Oh D, Kim S, Choi K-S, Kang D-H (2018) Cultivating *Spirulina maxima*: Innovative approaches. In: Tiwari A (Ed), Cyanobacteria. InTechOpen, 61–83. https://doi.org/10.5772/intechopen.74265

Kochert G (1978) Carbohydrate determination by the phenol-sulfuric acid method. In: Hellebust JA, Craigie JS (Eds) Handbook of Phycological Methods. Cambridge University Press, London, 95–97.

Kurpan D, Idà A, Körner FG, Bombelli P, da Silva Aguiar JP, Marinho LM, do Valle AF, Acién FG, Trasatti SP, Schievano A (2024) Long-term evaluation of productivity and harvesting efficiency of an industrial Spirulina (*Arthrospira platensis*) production facility. Bioresource Technology Reports 25: 101741. https://doi.org/10.1016/j.biteb.2023.101741

Lafarga T, Fernández-Sevilla JM, González-López C, Acién-Fernández FG (2020) *Spirulina* for the food and functional food industries. Food Research International 137: 109356. https://doi.org/10.1016/j.foodres.2020.109356

Li Z, Liu Y, Zhou T, Cao L, Cai Y, Wang Y, Cui X, Yan H, Ruan R, Zhang Q (2022) Effects of culture conditions on the performance of *Arthrospira platensis* and its production of exopolysaccharides. Foods 11: 2020. https://doi.org/10.3390/foods11142020

Lim P, Leaw C, Usup G, Kobiyama A, Koike K, Ogata T (2006) Effects of light and temperature on growth, nitrate uptake, and toxin production of two tropical dinoflagellates: *Alexandrium tamiyavanichii* and *Alexandrium minutum* (Dinophyceae). Journal of Phycology 42: 786–799. https://doi.org/10.1111/j.1529-8817.2006.00249.x

Lodi A, Binaghi L, Solisio C, Converti A, Del Borghi M (2003) Nitrate and phosphate removal by *Spirulina platensis*. Journal of Industrial Microbiology and Biotechnology 30: 656–660. https://doi.org/10.1007/s10295-003-0094-5

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Lu Y-M, Xiang W-Z, Wen Y-H (2011) *Spirulina* (*Arthrospira*) industry in inner Mongolia of China: current status and prospects. Journal of Applied Phycology 23: 265–269. https://doi.org/10.1007/s10811-010-9552-4

Menzel DW, Corwin N (1965) The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. Limnology and Oceanography 10: 280–282. https://doi.org/10.4319/lo.1965.10.2.0280

Morocho-Jácome AL, Mascioli GF, Sato S, de Carvalho JCM (2016) Evaluation of physicochemical treatment conditions for the reuse of a spent growth medium in *Arthrospira platensis* cultivation. Algal Research 13: 159–166. https://doi.org/10.1016/j.algal.2015.11.022

Myklestad S, Haug A (1972) Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. Journal of Experimental Marine Biology and Ecology 9(2): 125–136. https://doi.org/10.1016/0022-0981(72)90041-X

Pan-utai W, Kahapana W, Iamtham S (2018) Extraction of C-phycocyanin from *Arthrospira* (*Spirulina*) and its thermal stability with citric acid. Journal of Applied Phycology 30: 231–242. https://doi.org/10.1007/s10811-017-1155-x

Phélippé M, Gonçalves O, Thouand G, Cogne G, Laroche C (2019) Characterization of the polysaccharides chemical diversity of the cyanobacteria *Arthrospira platensis*. Algal Research 38: 101426. https://doi.org/10.1016/j.algal.2019.101426

Priyanka S, Varsha R, Riya Verma, Ayenampudi SB (2023) Spirulina: a spotlight on its nutraceutical properties and food processing applications. Journal of Microbiology, Biotechnology and Food Sciences 12(6): e4785. https://doi.org/10.55251/jmbfs.4785

Radmann EM, Reinehr CO, Costa JAV (2007) Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. Aquaculture 265: 118–126. https://doi.org/10.1016/j.aquaculture.2007.02.001

Ragusa I, Nardone GN, Zanatta S, Bertin W, Amadio E (2021) *Spirulina* for skin care: a bright blue future. Cosmetics 8: 7. https://doi.org/10.3390/cosmetics8010007

Salehipour-Bavarsad F, Nematollahi MA, Pistocchi R, Pezzolesi L (2024) Algal food safety: possible contaminations, challenges of harmonized quality assessments, and suggested recommendations for the nascent industry of microalgae-based products. Algal Research 81: 103579. https://doi.org/10.1016/j.algal.2024.103579

Soni RA, Sudhakar K, Rana RS (2017) *Spirulina* – From growth to nutritional product: a review. Trends in Food Science and Technology 69: 157–171. https://doi.org/10.1016/j.tifs.2017.09.010

Trabelsi L, Ben Ouada H, Bacha H, Ghoul M (2009) Combined effect of temperature and light intensity on growth and extracellular polymeric substance production by the cyanobacterium *Arthrospira platensis*. Journal of Applied Phycology 21: 405–412. https://doi.org/10.1007/s10811-008-9383-8

Xie Y, Jin Y, Zeng X, Chen J, Lu Y, Jing K (2015) Fed-batch strategy for enhancing cell growth and C-phycocyanin production of *Arthrospira* (*Spirulina*) *platensis* under phototrophic cultivation. Bioresource Technology 180: 281–287. https://doi.org/10.1016/j.biortech.2014.12.073

Yuan D, Yao M, Wang L, Li Y, Gong Y, Hu Q (2019) Effect of recycling the culture medium on biodiversity and population dynamics of bio-contaminants in *Spirulina platensis* mass culture systems. Algal Research 44: 101718. https://doi.org/10.1016/j.algal.2019.101718

Zarrouk C (1996) Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch et Gardner) Geitler. Ph.D Thesis, University of Paris, France.

Zerlotin M (2021) Produttività di *Arthrospira platensis* in un impianto industriale in open ponds. Master's thesis. University of Bologna, Italy.

Zhu CJ, Lee YK (1997) Determination of biomass dry weight of marine microalgae. Journal of Applied Phycology 9: 183–194 https://doi.org/10.1023/A:1007914806640